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# Role of Proliferating Cell Nuclear Antigen Interactions in the Mismatch Repair-Dependent Processing of Mitotic and Meiotic Recombination Intermediates in Yeast

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ABSTRACT

The mismatch repair (MMR) system is critical not only for the repair of DNA replication errors, but also for the regulation of mitotic and meiotic recombination processes. In a manner analogous to its ability to remove replication errors, the MMR system can remove mismatches in heteroduplex recombination intermediates to generate gene conversion events. Alternatively, such mismatches can trigger an MMR-dependent antirecombination activity that blocks the completion of recombination, thereby limiting interactions between diverged sequences. In *Saccharomyces cerevisiae*, the MMR proteins Msh3, Msh6, and Mlh1 interact with proliferating cell nuclear antigen (PCNA), and mutations that disrupt these interactions result in a mutator phenotype. In addition, some mutations in the PCNA-encoding *POL30* gene increase mutation rates in an MMR-dependent manner. In the current study, *pol30*, *mlh1*, and *msh6* mutants were used to examine whether MMR–PCNA interactions are similarly important during mitotic and meiotic recombination. We find that MMR–PCNA interactions are important for repairing mismatches formed during meiotic recombination, but play only a relatively minor role in regulating the fidelity of mitotic recombination.

THE failure to accurately replicate and repair genomic DNA leads to a wide variety of somatic and germ-line mutations, most of which are deleterious. Organisms thus have evolved multiple mechanisms to promote the stability of DNA and ensure faithful genome propagation. One of these mechanisms is the mismatch repair (MMR) system, best known for its role in correcting errors made by DNA polymerases during DNA replication (reviewed by Harfe and Jinks-Robertson 2000; Schofield and Hsieh 2003; Kunkel and Erie 2005). In addition to this replication-associated "spellchecker" function, the MMR system promotes genome stability via an antirecombination activity that prevents recombination between diverged sequences (reviewed by Surtees *et al.* 2004). Because such sequences are not identical, there is the potential for mismatches to be present within heteroduplex recombination intermediates. The MMR system recognizes such mismatches and prevents recombination from going to completion, thereby limiting detrimental genome rearrangements between dispersed repeated sequences. In some cases, a single mismatch within heteroduplex DNA is sufficient to reduce mitotic recombination in an MMR-dependent manner (Datta *et al.* 1997).

In addition to the spellchecker and antirecombination functions that promote mitotic genome stability, the MMR system is important during meiosis, specifically in meiotic recombination (reviewed by Hoffmann and Borts 2004; Surtees *et al.* 2004). As in mitosis, sequence divergence can trigger meiotic antirecombination activity of the MMR machinery, an activity thought to be important for enforcing homolog–homolog interactions and species barriers. Mismatches formed in meiotic recombination intermediates between different alleles, however, are more often simply repaired by the MMR system. Depending on which strand is used as the template for repair, either Mendelian segregation of the alleles will be restored or a gene conversion event will occur. In the nomenclature of eight-spored asci, restoration-type repair is manifested as 4:4 events while gene conversion results in 6:2 or 2:6 allele segregation. If MMR fails to correct a mismatch, the resulting meiotic product will have both alleles, resulting in a 5:3 or 3:5 ratio, referred to as postmeiotic segregation (PMS). Loss of MMR function typically results in an increase in PMS events and a concomitant reduction in gene conversion.

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Finally, in addition to mismatch correction, certain MMR proteins have also been shown to be involved in the processing of meiotic recombination intermediates to produce crossovers (Hunter and Borts 1997; Wang *et al.* 1999).

The mechanism of MMR associated with DNA replication is best understood in prokaryotes, where it involves the proteins MutS, MutL, and MutH (reviewed by Iyer *et al.* 2006; Joseph *et al.* 2006). MutS homodimers recognize errors made by DNA polymerase, binding to both base substitution and frameshift intermediates. MutH homodimers bind to nearby hemimethylated Dam sites, which mark the region as newly replicated and provide a mechanism for distinguishing the template and nascent strands. MutL homodimers promote an interaction between MutS and MutH, which activates MutH to nick the unmethylated, nascent strand. This nick provides an entry point for a helicase to unwind and exonucleases to remove the mismatch-containing region. Thus, MMR is nick directed, and importantly, repair is targeted specifically to the nascent strand.

In eukaryotes, the bacterial MutS homodimer is replaced by Msh (*M*ut*S h*omolog) protein heterodimers (reviewed by Kunkel and Erie 2005). MutSα is composed of Msh2 and Msh6 and broadly recognizes base/base mismatches and small-loop frameshift intermediates. MutSβ, which is a heterodimer of Msh2 and Msh3, recognizes small- and large-loop frameshift intermediates and may recognize specific base/base mismatches (Harrington and Kolodner 2007). MutSα and MutSβ interact primarily with the MutL-like heterodimer MutLα (composed of Mlh1 and Pms1) to correct mismatches. No MutH homolog has been found in eukaryotes, and the method of strand discrimination during the repair of replication errors is not fully understood. As in *Escherichia coli,* however, experiments using purified proteins or cell extracts have shown that a nick is sufficient to direct eukaryotic MMR (reviewed by Iyer *et al.* 2006). In addition to the nicks that are naturally present in nascent DNA, recent studies have demonstrated that human MutLα has the ability to introduce additional nicks into DNA *in vitro* (Kadyrov *et al.* 2006) and that disruption of the yeast MutLα endonuclease domain results in a mutator phenotype *in vivo* (Erdeniz *et al.* 2007; Kadyrov *et al.* 2007).

In addition to the classical MMR proteins, the *p*roliferating *c*ell *n*uclear *a*ntigen (PCNA) sliding clamp is important for the repair of replication errors. During replication, the PCNA homotrimer encircles duplex DNA and tethers the replication machinery to the template, thereby increasing DNA polymerase processivity. PCNA also acts as a landing pad for many other proteins involved in DNA metabolism (reviewed by Moldovan *et al.* 2007). The first indication of a role for PCNA in MMR was obtained in yeast, where some alleles of the PCNA-encoding *POL30* gene produce a mutator phenotype and cause instability of microsatellite repeats (Johnson *et al.* 1996; Umar *et al.* 1996). PCNA has since been shown to interact directly with the MutS homologs Msh3 and Msh6 (Umar *et al.* 1996; Flores-Rozas *et al.* 2000), as well as with the MutL homolog Mlh1 (Umar *et al.* 1996; Dzantiev *et al.* 2004; Lee and Alani 2006). Each of these three proteins contains a putative PCNA interaction domain called the PIP box (Warbrick 1998) which, when mutated, disrupts interaction with PCNA *in vitro* and confers a mutator phenotype *in vivo* (Kokoska *et al.* 1999; Clark *et al.* 2000; Flores-Rozas *et al.* 2000; Lee and Alani 2006). Additionally, *in vitro* experiments have shown that the inclusion of PCNA enhances the ability of MutSα to discriminate between DNAs containing mismatches and those that lack mismatches (Flores-Rozas *et al.* 2000). Finally, work done with human cell extracts has shown that a nick positioned 5′ of a mismatch requires only hMutSα, RPA, and hExo1 to direct subsequent strand removal, but that a nick 3′ to a mismatch additionally requires MutLα, PCNA, and replication factor C (RFC) for strand removal (Constantin *et al.* 2005).

While PCNA is clearly important in the MMR-directed repair of replication errors, it is not known if PCNA plays an equivalent role in the recombination-related functions of MMR. In spite of the basic similarities between mismatch recognition during replication and recombination, there are data suggesting fundamental differences between how the MMR system removes replication errors and how it affects recombination processes in *Saccharomyces cerevisiae.* For example, it appears that the MutL homologs, while essential for the repair of replication-generated mismatches, are not as important as the MutS homologs in some antirecombination processes (Chen and Jinks-Robertson 1999; Nicholson *et al.* 2000; Sugawara *et al.* 2004). In addition, separation-of-function alleles of *PMS1* have been identified that reduce the mitotic antirecombination activity of MutLα and the repair of mismatches formed during meiotic recombination, but have little or no effect on the repair of replication errors (Welz-Voegele *et al.* 2002). Mutant alleles of *MLH1* have also been identified that are deficient in the repair of mismatches formed during either replication or meiotic recombination, but not both (Argueso *et al.* 2002, 2003). Finally, a member of the RecQ family of DNA helicases, Sgs1, has been shown to be involved in the mitotic antirecombination function of MMR, but plays no known role in the spellchecker or meiotic recombination functions (Myung *et al.* 2001; Spell and Jinks-Robertson 2004b; Sugawara *et al.* 2004). Given these basic differences, it is important to determine if the mitotic role of PCNA is limited only to the spellchecker function of MMR, or if it is also required for the antirecombination activity. Similarly, it is important to determine if the role of PCNA in MMR is confined to mitotic MMR processes, or if it is necessary for the repair of mismatches formed during meiotic recombination as well. In this study, we examine the

effect of altering the MMR–PCNA interaction on MMR functions during both mitotic and meiotic recombination in yeast. Our results demonstrate that PCNA is important for the repair of mismatches in meiotic recombination intermediates but plays only a minor role in regulating mitotic recombination fidelity.

## MATERIALS AND METHODS

**Media and growth conditions:** For strain constructions, cells were grown in YEP medium (2% yeast extract and 4% peptone) supplemented with 2% dextrose and 0.25 g/liter adenine (YPD; 2.5% agar for plates). Drug selections were done on YPD containing either 300 μg/ml hygromycin or 200 μg/ml G418. For mitotic recombination and mutation-rate determinations in the SJR328-derived strains with mutant chromosomal alleles, cultures were grown in YEP supplemented with 4% galactose/2% glycerol (YEPGG) and 0.25 g/liter adenine; mutation rates in AS4- and AS13-derived strains were measured in YPD-grown cultures. Selection for $His^+$ recombinants was performed on synthetic medium supplemented with 2% galactose/2% glycerol/2% ethanol and a complete amino acid mix deficient in histidine (GGE −His). For mutation-rate analysis, cells were plated on arginine-deficient synthetic dextrose medium (SD −Arg) supplemented with 60 μg/ml canavanine. For SJR328-derived strains with plasmid-encoded *msh6* alleles, cells were grown in synthetic medium supplemented with 4% galactose/2% glycerol and deficient in leucine (SGG −Leu). $His^+$ and canavanine-resistant cells were selected as above except that leucine was omitted from the media. For the plasmid-containing strains, total cell numbers were determined on SD −Leu medium. For meiotic recombination studies, standard growth and sporulation conditions were used (Sherman 1991) except as noted.

**Plasmids:** Plasmid pSR559 was constructed by subcloning a *Kpn*I/*Not*I fragment containing the *pol30-52* allele from pBL241-52 (Ayyagari *et al.* 1995) into *Kpn*I/*Not*I-digested pRS306 (Sikorski and Hieter 1989). pSR873, which contains the *pol30-201,204* allele, was constructed by site-directed mutagenesis (QuickChange, Stratagene, La Jolla, CA) using the *pol30-201* plasmid pRDK925 (Lau *et al.* 2002) as a template and introducing the *pol30-204* mutation using primers pol30-204Fmut (5′-CCTCACTAAGTAAAATCCTACGTCGTGGTAAC AACACCGATACATTAACACTAATTGC-3′) and pol30-204Rmut (5′-GCAATTAGTGTTAATGTATCGGTGTTGTTACCACGACGT AGGATTTTACTTAGTGAGG-3′). The sequence change is underlined and creates an *Hpy*99I site.

**Strain constructions:** A complete list of yeast strains is given in Table 1. The mitotic antirecombination experiments were done in the SJR328 background (*MAT*α *ade2-101 his3Δ200 ura3-Nhe lys2ΔRV::hisG leu2-R*) with the cβ2-100% or cβ2-4ns constructs integrated at *LEU2* (Nicholson *et al.* 2000). The meiotic experiments were done using diploids obtained by mating appropriate derivatives of haploids AS4 (*MAT*α *arg4-17 trp1-1 tyr7-1 ade6 stp22 ura3*; Stapleton and Petes 1991) and AS13 (*MAT***a** *leu2-Bst ade6 ura3 rme1*; Stapleton and Petes 1991). In preparation for the introduction of chromosomal *pol30* or plasmid-encoded *msh6* alleles, $Leu^-$ derivatives of $Leu^+$ strains were constructed by replacing *LEU2* with a *leu2Δ::kanMX4* allele generated using pFA6-kanMX4 (Wach *et al.* 1994) as a template (forward primer 5′-ATGTCTGCCCC TAAGAAGATCGTCGTTTTGCCAGGTGACCACGTTGTCAA Gcagctgaagcttcgtacg and reverse primer 5′-TTAAGCAAGG ATTTTCTTAACTTCTTCGGCGACAGCATCACCGACTTCG GTGGaggccactagtggatctg; *LEU2*-homologous sequences are capitalized). The *leu2* derivative of HMY134 (SJR2705) was constructed by transforming HMY134 with a PCR-generated *leu2Δ::hyg* fragment. This fragment was produced using the same oligonucleotides described above to amplify *hphMX4* from plasmid pAG32 (Goldstein and McCusker 1999).

Strains containing the chromosomal *pol30-104, pol30-201, pol30-204,* or *pol30-201,204* allele were constructed by one-step allele replacement using a *Sac*I fragment derived from the appropriate plasmid and containing both the *pol30* allele and a *LEU2* marker [pCH1577 (Johnson *et al.* 1996), pRDK925, pRDK926 (Lau *et al.* 2002), or pSR873, respectively]. *pol30-52* strains were constructed via two-step allele replacement using either *Eco*RI-digested pSR559 or pBL241-52 (Ayyagari *et al.* 1995). The *mlh1-QLF* allele was introduced by transformation with an *mlh1-QLF:kanMX4* fragment derived from *Kpn*I-digested pEAA282 (Lee and Alani 2006), and the *mlh1Δ::hygMX2* allele was introduced using a PCR-derived fragment (forward primer 5′-TTTTGATACGATAGTGATAGTAAATGGAAGGT AAAAATAACATAGACCTATCAATAAGCAcagctgaagcttcgtacg and reverse primer 5′-CACAATCACACTCAGGAAATAAACA AAAAACTTTGGTATTACAGCCAAACGTTTTAAAGaggccact agtggatctg; *MLH1*-homologous sequences are capitalized). The presence of the *mlh1-QLF* and *pol30* alleles was inferred by phenotype (elevated mutation rates and cold sensitivity as appropriate) and confirmed by sequencing. For complementation analysis, low copy-number plasmids derived from the *CEN-LEU2* vector pRS315 (Sikorski and Hieter 1989) and containing wild-type (WT) or mutant *MSH6* alleles were transformed into various *msh6Δ leu2Δ* strains (Table 1). *MSH6* was contained on pRDK3572, *msh6Δ2-251* on pRDK4650, *msh6Δ51-251* on pRDK4715, and *msh6-FFAA,Δ51-251* on pRDK4758 (Shell *et al.* 2007). Transformants used for complementation analysis were selected and maintained on leucine-deficient medium.

The progenitor strains (indicated in parentheses) used in various constructions in the AS4- and AS13-derived strains were as follows: JSY162 (PD73), SJR2184 (PD73), SJR2203 (SJR2184), JSY332 (SJR2203), SJR2577 (AS4), and SJR2578 (PD73). The *msh2Δ::hygB* allele in JSY332 was introduced by transforming SJR2203 with a PCR fragment generated by amplification of the plasmid pAG32 (Goldstein and McCusker 1999) with the primers f *msh2-Δ* and r *msh2-Δ* (Kearney *et al.* 2001). *ARG4* derivatives were constructed of the strains AS4, RKY1721 (Alani *et al.* 1994), and JSY173 (JSY208, JSY203, and JSY209, respectively) by one-step transplacement with the 2.4-kb *Sal*I fragment derived from the plasmid pMW52 (White *et al.* 1993).

**Measuring mitotic recombination and mutation rates:** For strains in the SJR328 background, individual colonies were grown in 5 ml YEPGG or 10 ml SGG −Leu medium at 30° for 2 or 3 days, respectively; strains of the AS4 or AS13 background were grown for 1 day under the same conditions in YPD. The cells were harvested by centrifugation and washed with water. Appropriate dilutions were plated to select for $His^+$ recombinants or canavanine-resistant (Can-R) mutants, as well as on the appropriate nonselective medium to determine the total number of cells per culture. Colonies were counted after 2 days on YPD and SD or after 4 days on GGE media.

To calculate recombination and mutation rates, the median number of $His^+$ or Can-R colonies was determined using at least 12 independent cultures. The method of the median (Lea and Coulson 1949) was used to calculate recombination rates (number of events per cell per generation). Ninety-five percent confidence intervals (C.I.'s) were obtained by ranking the selective medium counts in ascending order (Spell and Jinks-Robertson 2004a), and then using Table B11 from Altman (1990) to determine which cultures provided the upper and lower limits of each C.I. Confidence limits on the ratio of homeologous to homologous recombination (HER:HR) were calculated using the 95% C.I.'s on individual

**TABLE 1**

**Yeast strains**

| Strain | Relevant genotype[a] | Source[b] |
|---|---|---|
| | SJR328-derived haploids | |
| GCY313 | cβ2-100% substrates | Nicholson *et al.* (2000) |
| GCY420 | cβ2-100%, *msh3Δ::hisG msh6Δ::hisG* | Nicholson *et al.* (2000) |
| SJR1906 | cβ2-100%, *pol30-201:LEU2* | This study |
| SJR2173 | cβ2-100%, *msh3Δ::hisG msh6Δ::hisG pol30-201:LEU2* | This study |
| SJR1896 | cβ2-100%, *pol30-204:LEU2* | This study |
| SJR2127 | cβ2-100%, *pol30-201,204:LEU2* | This study |
| SJR2084 | cβ2-100%, *pol30-104:LEU2* | This study |
| SJR2083 | cβ2-100%, *pol30-52* | This study |
| SJR2573 | cβ2-100%, *mlh1Δ::hygMX* | This study |
| SJR2575 | cβ2-100%, *mlh1-QLF:kanMX* | This study |
| SJR2690 | cβ2-100%, *msh6Δ::hisG* | SJR1856 transformed with pRS315 |
| SJR2691 | cβ2-100%, *msh6Δ::hisG/MSH6* | SJR1856 transformed with pRDK3572 |
| SJR2692 | cβ2-100%, *msh6Δ::hisG/msh6Δ2-251* | SJR1856 transformed with pRDK4650 |
| SJR2693 | cβ2-100%, *msh6Δ::hisG/msh6Δ51-251* | SJR1856 transformed with pRDK4715 |
| SJR2694 | cβ2-100%, *msh6Δ::hisG/msh6-FFAA,Δ51-251* | SJR1856 transformed with pRDK4758 |
| GCY562/615 | cβ2-4ns substrates | Nicholson *et al.* (2000) |
| GCY834 | cβ2-4ns, *msh3Δ::hisG msh6Δ::hisG* | Nicholson *et al.* (2000) |
| SJR1889 | cβ2-4ns, *pol30-204:LEU2* | This study |
| SJR1899 | cβ2-4ns, *pol30-201:LEU2* | This study |
| SJR2174 | cβ2-4ns, *msh3Δ::hisG msh6Δ::hisG pol30-201:LEU2* | This study |
| SJR2126 | cβ2-4ns, *pol30-201,204:LEU2* | This study |
| SJR2082 | cβ2-4ns, *pol30-104:LEU2* | This study |
| SJR2081 | cβ2-4ns, *pol30-52* | This study |
| SJR2574 | cβ2-4ns, *mlh1Δ::hygMX* | This study |
| SJR2576 | cβ2-4ns, *mlh1-QLF:kanMX* | This study |
| SJR2695 | cβ2-4ns, *msh6Δ::hisG* | SJR1850 transformed with pRS315 |
| SJR2696 | cβ2-4ns, *msh6Δ::hisG/MSH6* | SJR1850 transformed with pRDK3572 |
| SJR2697 | cβ2-4ns, *msh6Δ::hisG/msh6Δ2-251* | SJR1850 transformed with pRDK4650 |
| SJR2698 | cβ2-4ns, *msh6Δ::hisG/msh6Δ51-251* | SJR1850 transformed with pRDK4715 |
| SJR2699 | cβ2-4ns, *msh6Δ::hisG/msh6-FFAA,Δ51-251* | SJR1850 transformed with pRDK4758 |
| | AS4-derived haploids | |
| RKY1721 | *msh2::Tn10LUK7-7* | Alani *et al.* (1994) |
| JSY203 | *msh2::Tn10LUK7-7 ARG4* | This study |
| JSY208 | *ARG4* | This study |
| JSY173 | *pol30-52* | This study |
| JSY209 | *pol30-52 ARG4* | This study |
| SJR2183 | *leu2Δ::kanMX* | This study |
| SJR2202 | *leu2Δ::kanMX pol30-201:LEU2* | This study |
| JSY345 | *pol30-201:LEU2* | Spore derived from SJR2202 × JSY125 |
| JSY125[c] | *MAT***a** + pDJ173 | Stone and Petes (2006) |
| HMY104 | *msh2Δ::kanMX4* | Kearney *et al.* (2001) |
| JSY218 | *MAT***a** *msh2Δ::kanMX4* + pDJ173 | Spore derived from JSY125 × HMY104 |
| JSY352 | *msh2Δ::kanMX4 pol30-201:LEU2* | Spore derived from JSY218 × JSY345 |
| SJR2577 | *mlh1-QLF:kanMX* | This study |
| HMY134 | *msh6Δ::kanMX4* | Kearney *et al.* (2001) |
| SJR2705 | *msh6Δ::kanMX4 leu2Δ::hyg* | This study |
| | AS13-derived haploids | |
| PD73 | *his4-AAG* | Detloff *et al.* (1991) |
| PD98 | *his4-3133* | Detloff *et al.* (1992) |
| RKY1452 | *his4-AAG msh2::Tn10LUK7-7* | Alani *et al.* (1994) |
| JSY162 | *his4-AAG pol30-52* | This study |
| SJR2184 | *his4-AAG leu2Δ::kanMX* | This study |
| SJR2203 | *his4-AAG leu2Δ::kanMX pol30-201:LEU2* | This study |
| JSY222 | *his4-AAG msh2Δ::kanMX pol30-52* | Spore derived from JSY214 × JSY162 |
| JSY332 | *his4-AAG leu2Δ::kanMX pol30-201:LEU2 msh2Δ::hygB* | *msh2Δ::hygB* derivative of SJR2203 |

(*continued*)

**TABLE 1**

**(Continued)**

| Strain | Relevant genotype[a] | Source[b] |
|---|---|---|
| HMY101 | *msh2Δ::kanMX his4::U1.1a* | Kearney *et al.* (2001) |
| HMY131 | *MAT*α | Kearney *et al.* (2001) |
| JSY214 | *MAT*α *his4-AAG msh2Δ::kanMX* | Spore derived from HMY101 × JSY127 |
| JSY350 | *his4-AAG msh2Δ::hygB pol30-201:LEU2* | Spore derived from JSY332 × HMY131 |
| JSY351 | *MAT*α *pol30-201:LEU2* | Spore derived from JSY332 × HMY131 |
| JSY344 | *his4-AAG pol30-201:LEU2* | Spore derived from JSY127 × SJR2203 |
| JSY127 | *MAT*α *his4-AAG* | Stone and Petes (2006) |
| JSY355 | *his4-3133 pol30-201:LEU2* | Spore derived from JSY351 X PD98 |
| SJR2578 | *his4-AAG mlh1-QLF:kanMX* | This study |
| JSY144 | *his4-AAG msh6Δ::kanMX4* | Stone and Petes (2006) |
| SJR2506 | *his4-AAG msh6Δ::kanMX4/ MSH6* | JSY144 transformed with pRDK3572 |
| SJR2507 | *his4-AAG msh6Δ::kanMX4/ msh6-FFAA,Δ51-251* | JSY144 transformed with pRDK4758 |
| | Diploid strains | |
| PD83 | *his4-AAG/HIS4* | Detloff *et al.* (1991) |
| PD99 | *his4-3133/HIS4* | Detloff *et al.* (1992) |
| MW103 | *his4-Sal/HIS4* | Stone and Petes (2006) |
| JSY338 | *his4-51/his4-51 his4-AAG/HIS4* | Stone and Petes (2006) |
| No. 5 | *his4-AAG/HIS4 msh2::Tn10LUK7-7/ msh2::Tn10LUK7-7* | Alani *et al.* (1994) |
| JSY240 | *his4-Sal/HIS4 msh2Δ::kanMX/msh2Δ::kanMX* | Stone and Petes (2006) |
| JSY230 | *his4-Sal/HIS4 mlh1Δ::kanMX/mlh1Δ::hygB* | Stone and Petes (2006) |
| JSY343 | *his4-51/his4-51 his4-AAG/HIS4 msh2Δ::kanMX/msh2Δ::kanMX* | Stone and Petes (2006) |
| JSY175 | *his4-AAG/HIS4 pol30-52/pol30-52* | JSY173 × JSY162 |
| JSY346 | *his4-AAG/HIS4 pol30-201:LEU2/pol30-201:LEU2* | JSY345 × JSY344 |
| JSY354 | *his4-AAG/HIS4 msh2Δ::kanMX/mshΔ2::hygB pol30-201:LEU2/pol30-201:LEU2* | JSY352 × JSY350 |
| JSY356 | *his4-3133/HIS4 pol30-201:LEU/pol30-201:LEU2* | JSY345 × JSY355 |
| HMY95 | *his4-AAG/HIS4 mlh1Δ::kanMX4/mlh1Δ::URA3* | Welz-Voegele *et al.* (2002) |
| DB101 | *his4-AAG/HIS4 mlh1Δ::URA3/mlh1Δ::URA3* | Welz-Voegele *et al.* (2002) |
| SJR2577 × SJR2578 | *his4-AAG/HIS4 mlh1-QLF:kanMX/mlh1-QLF:kanMX* | This study |
| SJR2705 × SJR2706 | *HIS4/his4-AAG msh6Δ::kanMX4/msh6Δ::kanMX4* + plasmid-borne *MSH6* | This study |
| SJR2705 × SJR2707 | *HIS4/his4-AAG msh6Δ::kanMX4/msh6Δ::kanMX4* + plasmid-borne *msh6-FFAA,Δ51-251* | This study |

[a] All strains were derived from haploid strains SJR328 (*MAT*α *ade2-101 his3Δ200 ura3ΔNhe lys2ΔRV::hisG leu2-R*; Nicholson *et al.* 2000), AS4 (*MAT*α *arg4-17 trp1-1 tyr7-1 stp22 ade6 ura3*; Stapleton and Petes 1991), or AS13 (*MAT***a** *leu2-Bst ade6 ura3 rme1*; Stapleton and Petes 1991) by transformation or by crosses with isogenic strains. Only those markers that are different from the haploid progenitor strains are shown.

[b] In crosses, AS4- and AS13-derived strains are shown on the left and the right of the ×, respectively.

[c] The plasmid pDJ173 (*URA3 STP22*) allows diploid strains derived from AS4 to sporulate (Stone and Petes 2006).

homeologous and homologous recombination rates. To calculate the lower confidence limit for the ratio, we divided the lower value of the 95% C.I. for the homeologous rate by the upper value of the 95% C.I. for the homologous rate. The upper confidence limit for the ratio was calculated by dividing the upper limit for the homeologous rate by the lower limit for the homologous rate.

**Measuring meiotic recombination:** Diploid strains were sporulated at either 18° or 30° as indicated in the tables. Because some of the mutants confer a mitotic mutator phenotype that causes high levels of spore inviability, haploid strains were mated overnight at 30° and the resulting diploids were transferred directly to sporulation medium without prior purification. Following tetrad dissection onto YPD medium, spore colonies were replica plated to various synthetic media lacking the appropriate amino acid to check the segregation of markers. Spore colonies on SD −His or SD −Arg medium were examined microscopically to detect PMS.

In the experiments involving the plasmid-borne *MSH6* and *msh6-FFAA,Δ51-251* alleles, the *MAT***a** haploid strains with the plasmids (SJR2706 and SJR2707) were grown in SD −Leu medium at 30° overnight. The *MAT*α strain without the plasmid (SJR2705) was grown overnight on YPD at 30°. Strains were mated and transferred to sporulation medium as described above.

## RESULTS

Previous studies have shown that mutations within either the PCNA-binding (PIP box) domain of MMR proteins (Clark *et al.* 2000; Flores-Rozas *et al.* 2000; Lee and Alani 2006; Shell *et al.* 2007) or the PCNA-encoding *POL30* gene (Johnson *et al.* 1996; Umar *et al.* 1996; Kokoska *et al.* 1999; Lau *et al.* 2002) reduce the efficiency of replication-error removal. It has not been

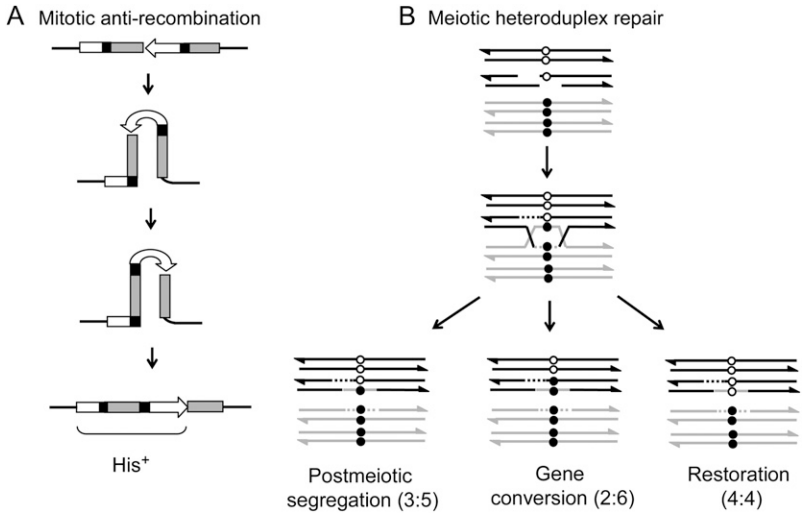


FIGURE 1.—Mitotic and meiotic recombination assays. (A) The mitotic antirecombination assay utilizes inverted repeats, each of which contains a recombination substrate composed of 350 bp of the chicken β-tubulin (cβ2) cDNA sequence (shaded boxes), the 5′ (or 3′) portion of an artificial intron (solid boxes), and the 5′ (or 3′) portion of the *HIS3* gene (open boxes). The recombination substrates used here were either identical (cβ2-100%) or contained four single-nucleotide differences (cβ2-4ns); heteroduplex recombination intermediates formed between the cβ2-4ns substrates contain up to four A/G or C/T mismatches. Recombination between the substrates results in reorientation of the 3′ and 5′ portions of *HIS3* gene, generating a full-length gene interrupted by a substrate-containing, functional intron. Although a crossover event is illustrated, reorientation can also occur via gene conversion between sister chromatids (CHEN and JINKS-ROBERTSON 1998). (B) The creation and repair of heteroduplex DNA during meiotic recombination. Open and solid circles represent WT and mutant alleles, respectively, and recombination is initiated by a double-strand break (DSB) on the WT chromosome. Following resection of the 5′ ends, DNA synthesis (dashed lines) initiated from an invading 3′ end displaces a D-loop. The heteroduplex DNA formed when the D-loop pairs with the unbroken chromosome contains a mismatch, as indicated by the pairing of open and solid circles. Failure to repair the mismatch results in a 3:5 PMS event; repair of the mismatch results in 2:6 gene conversion event or restoration of Mendelian segregation (4:4). Reciprocal 5:3 PMS and 6:2 gene conversion events can occur if the initiating DSB occurs on the mutant chromosome.

determined, however, if these mutations similarly affect the role of the MMR machinery in recombination-related processes. We thus examined the effects of mutations in *POL30*, *MLH1*, or *MSH6* on the MMR-dependent regulation of mitotic recombination fidelity and compared these effects to those seen in mutation-rate assays. Some alleles were additionally examined for their effects on the repair of mismatches in meiotic recombination intermediates.

The fidelity of mitotic recombination was examined using an inverted-repeat (IR) assay system (NICHOLSON *et al.* 2000). This system utilizes two cassettes oriented as IRs, each of which contains a 350-bp recombination substrate, a portion of the *HIS3* gene, and a segment of an artificial intron (the "IR-intron" system). The substrates were either 100% identical to one another (cβ2-100% "homologous" substrates) or differed by four evenly-spaced base substitutions (cβ2-4ns "homeologous" substrates; 99% identical). A recombination event between the substrates reorients the region between them and generates a functional *HIS3* gene, allowing cells to grow on medium lacking histidine (Figure 1A). While mutations in genes that have a general effect on recombination should affect homologous and homeologous recombination (HR and HER, respectively) to the same extent, those in genes that specifically regulate the fidelity of recombination will have a greater effect on the rate of HER than on the rate of HR, resulting in a ratio of HER:HR higher than that in a WT strain.

To examine the relevance of PCNA–MMR interactions to the repair of mismatches created during meiotic recombination (meiotic MMR), we sporulated diploid strains heterozygous for the *his4-AAG* and *arg4-17* alleles. The *his4-AAG* mutation is a T-to-A transversion at the second position of the *HIS4* start codon (DETLOFF *et al.* 1991) and the *arg4-17* mutation is a T-to-A transversion at position +127 of *ARG4* (WHITE *et al.* 1985). In both cases, heteroduplexes formed between the WT and mutant alleles create an A/A or T/T mismatch. These mismatches can be repaired to produce either a detectable gene conversion event (2 WT:6 mutant or 6 WT:2 mutant) or an undetectable restoration event (4 WT:4 mutant). If a mismatch fails to be repaired, the resulting spore colony will exhibit a sectored $His^+/His^-$ or $Arg^+/Arg^-$ phenotype. Such PMS events are manifested as either a 3 WT:5 mutant or a 5 mutant:3 WT segregation pattern in tetrads (Figure 1B). Other patterns of segregation in which more than one conversion and/or PMS event occur within a single tetrad are also observed, although such tetrads are much less frequent than tetrads with a single event (DETLOFF *et al.* 1991). The abundance of PMS tetrads relative to all classes of aberrant segregants reflects meiotic MMR efficiency.

**Regulation of mitotic recombination fidelity in MMR-defective *pol30* mutants:** Four of the *pol30* alleles used previously by others to demonstrate a role for PCNA in the repair of replication errors were used in our experiments to determine whether PCNA is important in the MMR-dependent regulation of mitotic recombination fidelity. Two of the alleles, *pol30-52* and *pol30-104*, confer microsatellite instability in an MMR-dependent manner (AYYAGARI *et al.* 1995; JOHNSON *et al.* 1996; KOKOSKA *et al.* 1999), but also have been shown to cause phenotypes not associated with MMR,

**TABLE 2**

***CAN1* forward mutation rates in *pol30* haploid strains**

| Background | Relevant genotype | Rate × $10^8$ | (C.I.) | Relative to WT |
|---|---|---|---|---|
| SJR328[a] | WT | 8.8 | (7.9–9.3) | 1.0 |
| | *msh3*Δ *msh6*Δ | 481 | (422–513) | 54.6 |
| | *pol30-52* | 1080 | (437–1400) | 123 |
| | *pol30-104* | 258 | (196–298) | 29.3 |
| | *pol30-201* | 68.8 | (62–121) | 7.8 |
| | *pol30-204* | 50.6 | (39.2–61.7) | 5.7 |
| | *pol30-201 msh3*Δ *msh6*Δ | 499 | (295–624) | 56.6 |
| | *pol30-201, 204* | 728 | (626–801) | 82.6 |
| AS13[b] | WT | 14.1 | (11.6–16.2) | 1.0 |
| | *msh2*Δ | 252 | (234–282) | 17.9 |
| | *pol30-52* | 908 | (707–1390) | 64.4 |
| | *msh2*Δ *pol30-52* | 4120 | (2400–5260) | 292 |
| | *pol30-201* | 173 | (149–195) | 12.3 |
| | *pol30-201 msh2*Δ | 342 | (325–394) | 24.3 |
| AS4[b] | WT | 11.7 | (11.0–16.5) | 1.0 |
| | *msh2*Δ | 193 | (179–209) | 16.5 |
| | *pol30-52* | 1290 | (857–1830) | 110 |

WT, wild type; C.I. = 95% confidence interval.

[a] Rates determined by combining data from the cβ2-100% and cβ2-4ns strains (see Table 1 for strain names).

[b] Strain names for the AS13 derivatives were PD73 (WT), RKY1452 (*msh2*Δ), JSY162 (*pol30-52*), JSY222 (*msh2*Δ *pol30-52*), SJR2203 (*pol30-201*), and JSY332 (*pol30-52 msh2*Δ); names for the AS4 derivatives were JSY208 (WT), JSY203 (*msh2*Δ), and JSY209 (*pol30-52*).

such as cell–cycle and replication defects (Ayyagari *et al.* 1995; Amin and Holm 1996; Johnson *et al.* 1996; Merrill and Holm 1998; Chen *et al.* 1999; Kokoska *et al.* 1999). The other two *pol30* alleles, *pol30-201* and *pol30-204*, were identified in a screen for PCNA mutations that increase mutation rate without causing the additional defects associated with the *pol30-52* and *pol30-104* alleles (Lau *et al.* 2002). In addition to measuring HR and HER rates, we examined the forward mutation rate at the *CAN1* locus (canavanine resistance, Can-R) in each *pol30* mutant to compare the associated mutator phenotypes in our strain backgrounds to those reported previously.

The effects of the *pol30* alleles in the forward mutation and mitotic recombination assays are presented in Tables 2 and 3, respectively, along with those conferred by a complete MMR deficiency (*msh3*Δ *msh6*Δ mutant). In the SJR328 background, elimination of MMR resulted in a 55-fold elevation in the Can-R rate. Although a slightly stronger mutator phenotype for the *pol30-104* than for the *pol30-52* allele was reported previously (Chen *et al.* 1999), in our experiments the *pol3-52* allele conferred a significantly stronger mutator phenotype than did the *pol30-104* allele (123- and 29-fold increases in Can-R relative to WT, respectively). Relative to the *pol30-52* and *pol30-104* alleles, the *pol30-201* and *pol30-204* alleles conferred only a modest mutator phenotype, with Can-R rates being elevated 8- and 6-fold, respectively. These *pol30-201* and *pol30-204* mutants appear to retain 85–90% of their MMR-related spellchecker activity in our strain background, a level >25–30% reported previously (Lau *et al.* 2002). The variability in relative Can-R rates in different strain backgrounds suggests that inherent genetic differences can modulate the magnitude of the mutator effects associated with specific PCNA mutations (see below as well).

The effects of the *pol30* alleles on mitotic recombination fidelity are best seen by dividing the HER rate by the HR rate and comparing this ratio to the HER:HR rate ratio in the WT control strain (Table 3). In the *msh3*Δ *msh6*Δ mutant, the HER:HR ratio was elevated 17-fold relative to that in a WT strain. Although both the *pol30-52* and *pol30-104* alleles conferred a significant increase in the HR rate (8- and 3-fold, respectively), consistent with a general replication defect, only the *pol30-52* allele conferred a slightly elevated HER:HR ratio (2-fold increase). The HR rates in both the *pol30-201* and *pol30-204* mutant were also significantly elevated relative to that in the WT strain (2- and 4-fold, respectively). We suggest that these mild hyperrecombination phenotypes may be indicative of a subtle, perhaps background-related, replication defect that was not apparent in the initial examination of these alleles (Lau *et al.* 2002). The 5-fold increase in HER associated with the *pol30-204* allele was similar to the 4-fold increase in HR, indicating no significant impairment in the efficiency of MMR-associated antirecombination. In contrast, in the *pol30-201* strain, recombination between the HER substrates was elevated significantly more than was recombination between the HR substrates (5- and 2-fold, respectively). In the complete absence of MMR (*msh3*Δ *msh6*Δ mutant), the presence of the *pol30-201* allele conferred no additional

**TABLE 3**

**Mitotic recombination rates between the HR (cβ2-100%) and HER (cβ2-4ns) substrates**

| Genotype | Recombination rate × $10^8$ | | | | HER:HR rate[a] | | |
|---|---|---|---|---|---|---|---|
| | HR | (C.I.) | HER | (C.I.) | | | |
| Wild type | 135 | (119–158) | 8.2 | (7.1–8.7) | 0.061 | (0.045–0.073) | 1.0 |
| *msh3*Δ *msh6*Δ | 251 | (206–283) | 266 | (227–290) | 1.1 | (0.8–1.4) | 17 |
| *pol30-52* | 1020 | (758–1490) | 131 | (115–305) | 0.13 | (0.077–0.40) | 2.1 |
| *pol30-104* | 432 | (306–680) | 25 | (15–30) | 0.058 | (0.02–0.098) | 0.95 |
| *pol30-201* | 225 | (174–364) | 42.3 | (36.3–52.2) | 0.19 | (0.10–0.30) | 3.1 |
| *pol30-204* | 535 | (389–578) | 42.2 | (35.8–53.2) | 0.079 | (0.062–0.14) | 1.3 |
| *pol30-201 msh3*Δ *msh6*Δ | 613 | (437–1000) | 382 | (348–473) | 0.62 | (0.35–1.1) | 10 |
| *pol30-201, 204* | 705 | (483–886) | 91.8 | (70.7–119) | 0.13 | (0.08–0.25) | 2.1 |

C.I. = 95% confidence interval.

[a] 99% confidence limits of the HER:HR ratios are shown in parentheses and were calculated as described in MATERIALS AND METHODS. The underlined numbers are the HER:HR rates normalized to wild type.

increase in the HER:HR ratio, consistent with a weak MMR-associated antirecombination activity for PCNA.

To determine the joint effect of the *pol30-201* and *pol30-204* mutations on the MMR-related roles of PCNA, we constructed the *pol30-201,204* double mutant allele. The Can-R rate of the *pol30-201,204* double mutant was 10-fold greater than that of either of the single-mutant strains and was similar to that observed in the MMR-defective (*msh3*Δ *msh6*Δ) background (Table 2). Thus, in terms of mutator phenotype, there appears to be a synergistic interaction between the two mutations when both are combined in the same protein. This synergism could reflect a complete loss of MMR or combined MMR and replication defects. In contrast, the 5-fold elevation in homologous recombination in the double mutant was very similar to the 4-fold elevation in the *pol30-204* single mutant. The HER:HR ratio in the double mutant was elevated 2-fold over that of the WT strain, a value slightly less than that obtained in the *pol30-201* single mutant and much less than that obtained in the *msh3*Δ *msh6*Δ background. The double-mutant data indicate that PCNA alterations can differentially affect the MMR-associated editing of replication errors and recombination intermediates.

**Repair of mismatches in meiotic heteroduplex DNA in *pol30* mutant strains:** The slightly elevated HER:HR ratios in the *pol30-52* and *pol30-201* mutants indicate that PCNA may play a minor role in the MMR-dependent regulation of mitotic recombination fidelity. These mutant alleles were introduced into diploid strains heterozygous for the *his4-AAG* and *arg4-17* alleles to examine PMS frequencies, the elevation of which would indicate a role for PCNA in meiosis-specific MMR. As in the SJR328 background used to monitor mitotic recombination fidelity, the mutator phenotypes conferred by the *pol30-52* and *pol30-201* alleles were determined in the haploid parents of the diploids used in the meiotic studies. In both the AS4- and AS13-derived *pol30-52* haploids, Can-R mutation rates were 3- to 6-fold higher than those observed in the corresponding MMR-defective (*msh2*Δ) mutants (Table 2). In addition, the *pol30-52 msh2*Δ AS13-derived strain had a Can-R mutation rate that was 4-fold higher than that of the *pol30-52* mutant (Table 2). Thus, as reported previously, the *pol30-52* allele elevates mutation rates in this genetic background in two ways: by reducing the efficiency of MMR and by elevating mutation rates by an MMR-independent mechanism (CHEN *et al.* 1999; KOKOSKA *et al.* 1999). In the AS13-derived haploid, *pol30-201* conferred a mutation rate that was only slightly less than that of an *msh2*Δ mutant, a phenotype much stronger than that observed in the SJR328 background, where 85% of MMR spellchecker activity was retained in the *pol30-201* mutant (Table 2). In the AS13 *msh2*Δ *pol30-201* background, the mutation rate was slightly higher than that observed in the single mutants, suggesting that the *pol30-201* allele may also generate mutations in an MMR-independent manner.

In AS4 × AS13-derived diploids, sporulation at 18° results in elevated frequencies of meiotic recombination at the *HIS4* locus (NAG and PETES 1993; FAN *et al.* 1995). *pol30-52* mutants are cold sensitive (AYYAGARI *et al.* 1995), however, and *pol30-52* diploids did not sporulate efficiently at either 18° or 25°. Because sporulation of the *pol30-52* diploid was most efficient at 30°, MMR-proficient (WT) and MMR-deficient (*msh2*Δ) strains were also sporulated at 30° for comparison. Sporulation of the WT strain at 30° resulted in a 3-fold decrease in the percentage of tetrads with aberrant segregation of *his4-AAG* relative to sporulation at 18° (Table 4), but did not significantly reduce the frequency of tetrads with aberrant segregation of *arg4-17* (Table 5). Compared to the WT strain, the *pol30-52* allele resulted in a significant decrease in the frequency of tetrads with aberrant segregation of arg*4-17* and a slight but not statistically significant decrease in tetrads with aberrant segregation of *his4-AAG*.

**TABLE 4**

**Meiotic segregation of the *his4-AAG* marker**

| Sporulation temperature | Strain | Relevant genotype | No. tetrads | Ab tetrads (%) | PMS tetrads (%) | PMS/Ab events (%) |
|---|---|---|---|---|---|---|
| 30° | PD83 | *WT* | 335 | 19 | 1.2 | 6.1 |
| | No. 5 | *msh2*Δ | 79 | 20 | 14 | 72 |
| | JSY175 | *pol30-52* | 74 | 14 | 4.1 | 30[d] |
| 18° | PD83[a] | *WT* | 482 | 58 | 10 | 18 |
| | No. 5[b] | *msh2*Δ | 111 | 64 | 55 | 90 |
| | JSY346 | *pol30-201* | 336 | 34 | 16 | 52[e] |
| | JSY354 | *pol30-201 msh2*Δ | 184 | 35 | 27 | 79[e] |
| | DB101/HMY95[a] | *mlh1*Δ | 465 | 57 | 49 | 89 |
| | SJR2577 × SJR2578 | *mlh1-QLF* | 226 | 51 | 38 | 78[f] |
| | SJR2705 × SJR2706[c] | *WT* | 165 | 63 | 21 | 30 |
| | SJR2705 × SJR2707[c] | *msh6-FFAA,*Δ*51-251* | 305 | 60 | 38 | 62[g] |

The columns of % events are as follows: % Ab tetrads, percentage of total tetrads with aberrant (non-4:4) segregation; % PMS tetrads, percentage of total tetrads with one or more PMS events, excluding tetrads with one gene conversion event and one PMS event (7:1, 1:7); % PMS/Ab events, percentage of aberrant events that are PMS events, calculated as described by Stone and Petes (2006). Two-tailed Fisher's exact tests were used to determine *P*-values and *P*-values were considered significant when $<0.05$. WT, wild type.

[a] Data from Welz-Voegele *et al.* (2002).

[b] Data from Kirkpatrick and Petes (1997).

[c] Alleles were encoded by the following plasmids: *MSH6* by pRDK3572 and *msh6-FFAA,*Δ*51-251* by pRDK4758 (Shell *et al.* 2007). To ensure that the plasmid was present during meiosis, only those tetrads that had at least one $Leu^+$ spore were scored.

[d] Significant increase from PD83 ($P = 0.04$) and significant decrease from JSY200/No. 5 ($P = 0.05$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[e] Significant increase from PD83 ($P < 0.001$) and significant decrease from JSY200/No. 5 ($P < 0.001$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[f] Significant increase from PD83 ($P < 0.001$) and significant decrease from DBY101/HMY95 ($P = 0.003$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[g] Significant increase from the WT SJR2705 × SJR2706 control ($P < 0.001$).

The efficiency of meiotic MMR is commonly expressed as the ratio of the number of PMS tetrads to the total number of aberrant tetrads (PMS + gene conversion tetrads). Because of the high level of meiotic recombination in the AS4 × AS13 diploids, however, a single tetrad may contain multiple aberrant events. To more accurately reflect MMR efficiency in these diploids, we calculated the ratio of total PMS events to total aberrant events (see Stone and Petes 2006 for an explanation of the calculation). We previously reported that there was a low level of PMS among total aberrant events for the *his4-AAG* mismatch in the WT strain sporulated at 18° (Detloff *et al.* 1991; Stone and Petes 2006); the percentage of PMS/total aberrant events was 18% (Table 4). At 30°, this ratio was even lower (6%; Table 4). In the *pol30-52* mutant strain sporulated at 30°, 30% of the aberrant events were PMS events (Table 4). This significant increase in PMS events relative to that observed in WT demonstrates that the *pol30-52* mutation reduces the efficiency of meiotic repair of mismatches in recombination intermediates. The ratio of PMS to total aberrant events observed for the *pol30-52* strain was only about half of the ratio found in MMR-deficient *msh2*Δ strain, however, indicating that the *pol30-52* mutation reduces but does not eliminate meiotic MMR. For the *arg4-17* marker, a significant reduction in the percentage of aberrant tetrads in the *pol30-52* diploid (from 9.6% in WT to 1.4%) made it difficult to determine the effect of the *pol30-52* mutation on PMS. Of the 74 complete tetrads examined, however, the one that was aberrant was also a PMS tetrad, whereas no PMS events were observed in the 32 aberrant tetrads from the WT strain.

In contrast to the *pol30-52* allele, the *pol30-201* allele does not confer cold sensitivity, thus allowing examination of the *pol30-201* diploid after sporulation at 18°. The *pol30-201* diploid had a lower level of aberrant segregation events at *HIS4* relative to the WT strain (Table 4) but the frequency of aberrant segregation at the *ARG4* locus was not affected by *pol30-201* (Table 5). The efficiency of MMR, as measured by the elevated PMS/aberrant events ratio, for both the *his4-AAG* and *arg4-17* mismatches was significantly reduced by *pol30-201*, with the effect on the *his4-AAG* mismatch being more severe. For the *his4-AAG* mismatch, the PMS/aberrant events ratio was ~60% of that observed in the *msh2*Δ strain; for *arg4-17* the ratio was only 25% of that in the *msh2*Δ strain.

The decrease in aberrant segregation of the *his4-AAG* marker in the *pol30-201* diploid could be due to a reduction in either the initiation of recombination at *HIS4* or the subsequent extension of heteroduplex DNA. Shortening of meiotic heteroduplexes can be

**TABLE 5**

**Meiotic segregation patterns of the *arg4-17* marker**

| Sporulation temperature | Strain | Relevant genotype | No. tetrads | Ab tetrads (%) | PMS tetrads[b] (%) | PMS/Ab events (%) |
|---|---|---|---|---|---|---|
| 30° | PD83 | WT | 335 | 9.6 | 0 | 0 |
| | No. 5 | *msh2*Δ | 79 | 8.9 | 5 | 57 |
| | JSY175 | *pol30-52* | 74 | 1.4 | 1.4 | 100[c] |
| 18° | MW103/JSY338[a] | WT | 742 | 7.5 | 0.1 | 1.7 |
| | JSY240/JSY343[a] | *msh2*Δ | 502 | 16 | 11 | 67 |
| | JSY346 | *pol30-201* | 336 | 8.6 | 1.5 | 16[d] |
| | JSY354 | *pol30-201 msh2*Δ | 184 | 11 | 3.8 | 35[e] |
| | JSY230 | *mlh1*Δ | 192 | 13 | 6.8 | 50 |
| | SJR2577 × SJR2578 | *mlh1-QLF* | 226 | 20 | 10 | 50[f] |
| | SJR2705 × SJR2706[b] | *WT* | 165 | 13 | 1.2 | 9.1 |
| | SJR2705 × SJR2707[b] | *msh6-FFAA,*Δ*51-251* | 305 | 14 | 1.6 | 11[g] |

The columns of % events are as follows: % Ab tetrads, percentage of total tetrads with aberrant (non-4:4) segregation; % PMS tetrads, percentage of total tetrads with one or more PMS events, excluding tetrads with one gene conversion event and one PMS event (7:1, 1:7); % PMS/Ab events, percentage of aberrant events that are PMS events, calculated as described by Stone and Petes (2006). Two-tailed Fisher's exact tests were used to determine *P*-values and *P*-values were considered significant when $<0.05$. WT, wild type.

[a] Data from Stone and Petes (2006). MW103/JSY338 and [JSY240/JSY343, JSY230] are isogenic to PD83 and No. 5, respectively, except for mutations at the *HIS4* locus, which have no significant effect on segregation of the *arg4-17* marker. Data from MW103/JSY338, JSY240/JSY343, and JSY230 are shown because spore colonies from these strains were examined microscopically for small sectors on medium lacking arginine, while spore colonies from 18° sporulations of PD83 and No. 5 were not.

[b] Alleles were encoded by the following plasmids: *MSH6* by pRDK3572 and *msh6-FFAA,*Δ*51-251* by pRDK4758 (Shell *et al.* 2007). To ensure that the plasmid was present during meiosis, only those tetrads that had at least one $Leu^+$ spore were scored.

[c] Significant increase from PD83 ($P = 0.03$) but no significant difference from No. 5 ($P = 1$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[d] Significant increase from MW103/JSY338 ($P = 0.02$) and significant decrease from JSY240/JSY343 ($P < 0.001$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[e] Significant increase from MW103/JSY338 ($P < 0.001$), significant decrease from JSY240/JSY343 ($P = 0.01$), and no significant change from JSY346 ($P = 0.18$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[f] Significant increase from MW103/JSY338 ($P < 0.001$) and no significant change from JSY230 ($P = 1$) in the number of PMS *vs.* the number of non-PMS aberrant events.

[g] No significant difference from SJR2705 × SJR2706 ($P = 1$).

detected genetically by examining the frequency of aberrant segregation as a function of distance from an initiating double-strand break (DSB). The aberrant segregation of markers far from the DSB will be greatly reduced compared to WT, while there will be less of an effect on segregation of markers located close to the DSB site. We thus examined the effect of the *pol30-201* mutation on segregation of the *his4-3133* marker, a poorly repaired marker that is located >2 kb farther from the *HIS4* DSB site than the *his4-AAG* marker (Detloff *et al.* 1992). There was 37% aberrant segregation (126/344 tetrads) for the *his4-3133* marker in the WT strain PD99 (Detloff *et al.* 1992), but only 9.4% aberrant segregation (27/288 tetrads) for this marker in the *pol30-201* mutant strain JSY356. This difference corresponds to a 75% reduction relative to the WT level of aberrant segregation for *his4-3133*. Because the level of aberrant segregation of the *his4-AAG* marker was reduced by only 40% in the *pol30-201* strain (Table 4), these data are consistent with a shorter average length of meiotic heteroduplexes in the *pol30-201* strain. Also consistent with shortened meiotic heteroduplexes in *pol30-201* strains was a 20–40% reduction in crossovers in each of three genetic intervals examined on chromosome III (data not shown). An association between shortened meiotic heteroduplexes and reduced crossing over has previously been documented in strains with a mutant DNA polymerase δ (*pol3-ct*; Maloisel *et al.* 2004).

**Effect of perturbing the Mlh1–PCNA interaction on MMR functions during recombination:** Mlh1 interacts physically with PCNA and contains an amino acid sequence related to the PIP box that is required for this interaction in *in vitro* assays (Lee and Alani 2006). Because mutation of this region of Mlh1 (the *mlh1-Q572A,L575A,F578A* or *mlh1-QLF* allele) confers a complete MMR defect in a frameshift reversion assay (Lee and Alani 2006), we examined its effect on the MMR-related spellchecker and antirecombination functions in the SJR328 background. As reported for the repair of frameshift intermediates, the *mlh1-QLF* allele resulted in a Can-R mutation rate indistinguishable from that of an *mlh1*Δ strain (Table 6). With diverged substrates in the IR-intron system, we consistently have observed that ~50% of the MMR-associated antirecombination activity persists in *pms1*Δ or *mlh1*Δ strains,

**TABLE 6**

**Mutagenesis and mitotic recombination in strains with PCNA interaction-defective alleles of *MLH1* and *MSH6***

| | *CAN1* forward mutation[a] | | | Recombination rate × $10^8$ | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Relevant genotype | Rate × $10^8$ | (C.I.) | Relative to WT | HR | (C.I.) | HER | (C.I.) | HER:HR rate[b] | |
| WT | 11.0 | (7.2–13.6) | 1.0 | 213 | (198–232) | 16.6 | (14.4–19.3) | 0.078 (0.062–0.98) | 1.0 |
| *mlh1*Δ | 1110 | (768–1480) | 101 | 222 | (169–306) | 102 | (56.2–159) | 0.46 (0.18–0.94) | 5.9 |
| *mlh1-QLF* | 508 | (444–818) | 57.7 | 192 | (144–208) | 54.4 | (43.9–64.5) | 0.28 (0.21–0.45) | 3.6 |
| Plasmid-encoded allele in *msh6*Δ strains[c] | | | | | | | | | |
| *MSH6* | 17.3 | (13.4–18.6) | 1.0 | 91.3 | (78.8–99.3) | 7.67 | (6.31–9.48) | 0.084 (0.064–0.12) | 1.0 |
| None | 973 | (765–1160) | 56.2 | 108 | (92.8–131) | 75.9 | (66.3–96.1) | 0.70 (0.51–1.0) | 8.3 |
| *msh6*Δ*2-251* | 402 | (272–466) | 23.2 | 92.2 | (73.5–106) | 13.5 | (11.9–15.7) | 0.15 (0.11–0.21) | 1.8 |
| *msh6*Δ*51-251* | 37.2 | (21.5–40.0) | 2.15 | 103 | (68.0–135) | 9.50 | (8.00–14.2) | 0.092 (0.059–0.21) | 1.1 |
| *msh6-FFAA,*Δ*51-251* | 236 | (151–279) | 13.6 | 135 | (96.3–151) | 11.5 | (9.30–15.6) | 0.085 (0.062–0.16) | 1.0 |

C.I. = 95% confidence interval; WT, wild type.

[a] The Can-R rates were calculated by pooling data from the HR and HER strains of a given genotype.

[b] Ninety-nine percent confidence limits of the HER:HR ratios are shown in parentheses and were calculated as described in MATERIALS AND METHODS. The underlined numbers are the HER:HR rates normalized to WT.

[c] Alleles were encoded by the following plasmids: *MSH6* by pRDK3572, *msh6*Δ*2-251* by pRDK4650, *msh6*Δ*51-251* by pRDK4715, and *msh6-FFAA,*Δ*51-251* by pRDK4758 (SHELL *et al.* 2007). HR and HER rates were measured in the SJR1856 and SJR1850 strain backgrounds, respectively.

indicating that the yeast MutS homologs can function independently of the major MutL heterodimer in this assay (CHEN and JINKS-ROBERTSON 1999; NICHOLSON *et al.* 2000; SPELL and JINKS-ROBERTSON 2003). In agreement with this observation, there was a 6-fold increase in the HER:HR ratio in the *mlh1*Δ relative to the WT strain (Table 6), compared to a 17-fold increase in this ratio in the *msh3*Δ *msh6*Δ mutant (Table 3). In the *mlh1-QLF* mutant, the HER:HR ratio was elevated 4-fold relative to the WT ratio. Importantly, the HER rates did not differ statistically in the *mlh1*Δ and *mlh1-QLF* mutants.

As with the *pol30* alleles that confer mitotic MMR defects, we also examined the effects of the *mlh1-QLF* allele on meiotic segregation of the *his4-AAG* and *arg4-17* alleles (Tables 4 and 5, respectively). In the *mlh1*Δ strain, 89% of the *his4-AAG* aberrant segregation events were PMS events compared to only 18% PMS events in the WT strain. There also was a strong elevation in PMS events in the *mlh1-QLF* mutant, with 78% of the aberrant events being PMS events. With the *arg4-17* allele, 50% of the aberrant events were PMS events in both the *mlh1*Δ and *mlh1-QLF* strains, compared to only 1.7% PMS events in the WT control strain.

**Recombination-related effects of *MSH6* alleles that disrupt interaction with PCNA:** Although the only defect of Mlh1-QLF protein detected *in vitro* is its interaction with PCNA (LEE and ALANI 2006), it is possible that this property may not accurately reflect the molecular defect *in vivo*. First, it is unclear whether the region of Mlh1 containing the QLF residues is available for potential interaction with PCNA. Although the crystal structure of a bacterial MutL fragment suggests that this region would be on the surface of the eukaryotic MutLα heterodimer (GUARNE *et al.* 2004), there is a report that this region is buried at the heterodimer interface (KOSINSKI *et al.* 2005). Second, the interaction of Mlh1 with PCNA described *in vitro* is not only very weak relative to the Msh6–PCNA interaction, but it involves electrostatic rather than the typical hydrophobic interactions (LEE and ALANI 2006). Because of uncertainty of the molecular defect associated with the Mlh1-QLF protein, we also examined alleles of *MSH6* that perturb its interaction with PCNA.

The Msh6 protein has a consensus PIP box that is required for interaction with PCNA in *in vitro* assays, but mutation of only the PIP box (*msh6-F33AF34A* allele, abbreviated here as *msh6-FFAA*) has only subtle effects on mutation rates (FLORES-ROZAS *et al.* 2000) and did not detectably affect antirecombination activity in the IR-intron assay (data not shown). A recent analysis of the Msh6–PCNA interaction indicates that the unstructured N terminus of Msh6 forms an extended tether to PCNA, and *msh6* alleles that are additionally missing this region have a much stronger phenotype than those that simply have a mutated PIP box (SHELL *et al.* 2007). We thus examined the ability of three plasmid-encoded *msh6* alleles to complement the mitotic phenotypes of an *msh6*Δ strain: *msh6*Δ*2-251*, *msh6*Δ*51-251*, and *msh6-FFAA,* Δ*51-251* (SHELL *et al.* 2007). Whereas the *msh6*Δ*51-251* allele produced a weak mutator phenotype similar to that of an *msh6-FFAA* allele (data not shown), the *msh6-FFAA,*Δ*51-251* allele with both mutations as well as the *msh6*Δ*2-251* allele produced strong mutator phenotypes in the *CAN1* assay (Table 6). Our mutator results are completely consistent with those reported by SHELL *et al.* (2007). In the *msh6*Δ control strain, the HER:HR rate ratio was elevated 8.3-fold (Table 6), which, as reported previously, is 2-fold less than that typically seen in *msh3*Δ *msh6*Δ or *msh2*Δ mutants (NICHOLSON *et al.* 2000; SPELL

and Jinks-Robertson 2003). Only the *msh6Δ2-251* allele elevated the HER:HR ratio a very modest 1.8-fold. Of particular significance, the Msh6-FFAA,Δ51-251 protein, which had lost substantial MMR function in the *CAN1* forward mutation assay (14-fold elevation in Can-R rate relative to a 56-fold elevation in the absence of Msh6), retained much, if not all, antirecombination activity.

The mitotic behavior of the *msh6-FFAA,Δ51-251* allele suggests that the antirecombination and spellchecker functions of the encoded protein may be separable. To explore this further, we analyzed the repair of meiotic heteroduplexes in an *msh6Δ/msh6Δ* diploid containing either an *MSH6* or an *msh6-FFAA,Δ51-251* allele on a complementing *LEU2/CEN* plasmid (Table 4). Because of plasmid stability issues, only those tetrads that produced at least one $Leu^+$ spore were included in the analyses of heteroduplex repair. Even so, PMS of the *his4-AAG* allele was elevated in the Msh6-complemented control strain relative to a diploid with a chromosomal *MSH6* allele (30% *vs.* 18% PMS/aberrant events). In the strain with the complementing *msh6-FFAA,Δ51-251* allele, the level of PMS was further elevated to 62%, demonstrating a clear and substantial loss of the meiotic mismatch repair activity of the encoded protein.

## DISCUSSION

The yeast MMR system regulates genome stability by editing the fidelity of DNA replication and homologous recombination. In addition to the canonical MutS and MutL homologs, PCNA is important for MMR-associated spellchecker activity, as mutations that disrupt physical interaction with Msh3, Msh6, or Mlh1 result in a mutator phenotype (Clark *et al.* 2000; Flores-Rozas *et al.* 2000; Lee and Alani 2006; Shell *et al.* 2007). Although the precise role of PCNA during replication-associated MMR is not clear, there are three general, nonmutually exclusive ways in which it may be important. First, the localization of PCNA to the replication fork provides a mechanism to concentrate MMR proteins to newly replicated DNA and thereby enhance the efficiency of error recognition. In addition, because of the directional orientation of the PCNA ring with respect to the template and nascent strands, PCNA may also provide a signal for strand discrimination (Umar *et al.* 1996). Second, *in vitro* binding assays have shown that "free" PCNA enhances the ability of MMR complexes to distinguish between DNA molecules with mismatches and those that lack mismatches (Flores-Rozas *et al.* 2000). Although the relevance of this observation to *in vivo* MMR is uncertain, it should be noted that PCNA–MMR interactions are important in the repair of mismatches engineered into transforming plasmids (Lau *et al.* 2002). Whether this repair occurs prior to the initiation of plasmid replication is not known. Third, because proteins that are sequentially involved in MMR (*e.g.*, Msh3/6, Mlh1, and Exo1) interact with PCNA, PCNA may coordinate the overall repair process by a protein hand-off mechanism (Lee and Alani 2006).

In all current models of recombination strand invasion is followed by replicative extension of the invading 3′ end (for a review see Krogh and Symington 2004), and hence one might expect PCNA to be present at an early stage. Because most mismatches in recombination intermediates would be formed via strand invasion (and possibly branch migration) rather than as a result of replication, however, it is not obvious whether PCNA might be similarly required for mismatch repair and/or antirecombination. It is possible, for example, that mismatch recognition and processing occurs at the initial strand-invasion step, before any DNA synthesis occurs. In addition, although repair directed to the newly synthesized DNA strand is essential for the spellchecker function of MMR, unbiased repair of mismatches generated during recombination would have relatively minor biological consequences. In the current study, we have specifically addressed the role of PCNA in the recombination-related processing of mismatches in both mitotic and meiotic recombination intermediates.

The fate of mismatches in meiotic heteroduplex DNA was assessed by tetrad dissection, where a decrease in the efficiency of mismatch repair leads to an increase in the ratio of PMS to total aberrant events. It should be noted that, as in the case of replication errors, the repair of meiotic heteroduplex DNA presumably involves mismatch excision followed by DNA synthesis to fill in the resulting gap. In the case of spontaneous mitotic recombination, it is not possible to directly study mismatch correction in an analogous manner. We thus used a mitotic antirecombination assay in which the production of recombinants between diverged sequences is strongly inhibited by the yeast MutS and MutL complexes. Because only antirecombination is dependent on the helicase activity of Sgs1 (Myung *et al.* 2001; Spell and Jinks-Robertson 2004b), its mechanism may be distinct from the nucleolytic destruction that characterizes the removal of replication errors. One can imagine scenarios in which MMR–PCNA interactions might be relevant to all, some, or none of the recombination processes involving mismatched heteroduplex DNA. As discussed in detail below, our data demonstrate that PCNA interactions are required for efficient MMR during meiotic recombination processes, but play a relatively minor role during mitotic antirecombination.

**Some mutator alleles of PCNA are weakly defective in the processing of recombination intermediates:** PCNA was first implicated in MMR through the analysis of the *pol30-104* (Johnson *et al.* 1996) and *pol30-52* (Umar *et al.* 1996) alleles, each of which additionally confers cold and mutagen sensitivity. Although teasing apart the contributions of general replication problems and specific MMR defects to mutagenesis has been

difficult in the corresponding mutants, they are generally assumed to be at least partially MMR defective. In our mitotic analyses, both alleles caused a significant hyperrecombination phenotype between identical substrates, underscoring the need to include homologous substrates as a control when examining HER. Whereas the *pol30-104* allele did not elevate HER to a greater extent than HR, the HER:HR ratio was elevated a modest twofold in the *pol30-52* mutant. The effect of the *pol30-52* allele on the repair of meiotic mismatches was much more striking, with the percentage of *his4-AAG* PMS events among aberrant events (hereafter referred to as % PMS) increasing fivefold, from 6.1% in the WT strain to 30% in the mutant. Even so, this increase in PMS was still significantly below the 72% observed in an *msh2*Δ strain.

To clarify the role of PCNA in MMR-related recombination processes, we also examined two *pol30* alleles whose only known defect is in MMR: *pol30-201* and *pol30-204* (Lau *et al.* 2002). Both alleles caused significant increases in mitotic HR (1.7- and 4.0-fold increases for *pol30-201* and *pol30-204*, respectively), however, and we suggest that each likely confers a subtle replication defect. The large hyperrec phenotype of the *pol30-204* strain relative to the *pol30-201* strain is consistent with the observation that *pol3-204*, but not *pol30-201*, mutants are slightly sensitive to MMS and UV (Lau *et al.* 2002). With respect to possible non-MMR defects in the *pol30-201* mutant, we found that this allele also resulted in a significant reduction in aberrant segregation of the *his4-AAG* marker (58% for WT, 64% for *msh2*Δ, and 34% for *pol30-201*). The reduction in aberrant segregation was even more severe for the *his4-3133* allele, which is located 2 kb farther from the site of recombination initiation than the *his4-AAG* marker, suggesting that heteroduplex extension is reduced in the *pol30-201* mutant. Together our mitotic and meiotic recombination analyses indicate a subtle impairment of DNA synthesis in the *pol30-201* mutant, at least in the strain backgrounds used in this study. On the basis of the large number of processes in which PCNA participates and the fact that many of the relevant proteins interact with the interdomain connector loop of PCNA (Moldovan *et al.* 2007), we suggest that it may not be possible to specifically perturb one DNA metabolic process through changes in PCNA without having collateral effects on other processes.

In the mitotic antirecombination assay, the *pol30-201*, but not the *pol30-204*, allele significantly elevated the HER:HR ratio (3-fold), but to a much lesser extent than did the complete elimination of MMR (17-fold). The *pol30-201* allele also reduced, but did not eliminate, the repair of mismatches in meiotic recombination intermediates (the *pol30-204* allele was not analyzed), with the % PMS being elevated for both the *his4-AAG* and *arg4-17* alleles (Tables 4 and 5). As with the *pol30-52* allele, it should be noted that the meiotic defect associated with the *pol30-201* allele was more pronounced than the mitotic defect. The observation that effects of the *pol30* alleles on meiotic and especially mitotic recombination processes were less than those associated with the complete loss of MMR may reflect some degree of continued function of the mutant PCNAs. Alternatively, PCNA might simply increase the efficiency of, but not be absolutely required for, the MMR-dependent regulation of recombination fidelity and meiotic heteroduplex repair. Although the data obtained with the *pol30-52* and *pol30-201* mutants are suggestive of a minor role for PCNA in the MMR-dependent regulation of recombination, the additional DNA metabolic defects conferred by all available *pol30* alleles temper the conclusions that can be drawn. For this reason, we additionally examined mutations in MMR proteins that are thought to specifically disrupt interactions with PCNA.

**MMR-dependent processing of recombination intermediates is abolished in *mlh1-QLF* mutants:** Alteration of the canonical Msh6 PIP box sequence (*msh6-FFAA* allele) causes only a weak mutator phenotype (Flores-Rozas *et al.* 2000) while that of the putative Mlh1 PIP-like domain (the *mlh1-QLF* allele) completely eliminates the spellchecker activity of the MMR machinery (Lee and Alani 2006; Table 2). Consistent with the relative mutator phenotypes of *msh6-FFAA* and *mlh1-QLF* strains, a PIP box-defective *msh6* allele did not detectably impair the antirecombination activity of the MMR machinery (data not shown) while the *mlh1-QLF* allele was indistinguishable from an *mlh1* null allele in terms of antirecombination activity (Table 6) and meiotic heteroduplex repair (Tables 4 and 5).

The interaction of MutLα and MutSα with PCNA was recently examined using surface plasmon resonance. Not only was the MutSα interaction with PCNA much stronger, but the salt resistances of the PCNA-containing complexes indicated that the MutLα interaction is largely ionic whereas the MutSα interaction is hydrophobic (Lee and Alani 2006). These different strengths and modes of interaction would not be expected if Mlh1, like Msh6, interacts with PCNA via its PIP-related domain. Finally, there is debate in the literature as to whether the region of Mlh1 defined by the *mlh1-QLF* allele would even be available for interaction with PCNA (Kosinski *et al.* 2005, but also see Clark *et al.* 2007). Given these considerations, it is not clear whether the phenotype conferred by mutating the PIP-related region of Mlh1 indeed reflects a specific PCNA-interaction defect, or whether the *mlh1-QLF* allele is MMR defective for an unrelated, uncharacterized reason. Additional data obtained with defined *msh6* alleles and discussed below suggest that the latter may be more likely.

**Relevance of the PCNA interaction to the recombination-related roles of Msh6:** Recent data suggest that many PIP-domain-containing proteins are tethered to PCNA via an unstructured, flexible linker region (Shell

*et al.* 2007). In the case of Msh6, this linker corresponds roughly to the region between the PIP box at the amino terminus and the DNA-binding domain, which is the first region of high homology between MutS proteins. Combining a PIP-box mutation with an internal deletion that shortens the Msh6 linker (*msh6-FFAA,Δ51-251* allele) confers a strong mutator phenotype, whereas the individual mutations produce only a weak phenotype (Shell *et al.* 2007). Given the caveats associated with MMR-defective *pol30* alleles and with the *mlh1-QLF* allele, the double mutant *msh6-FFAA,Δ51-251* allele may provide the most direct test for an involvement of PCNA in the recombination-related functions of the yeast MMR machinery. While the clear elevation in PMS frequency indicates defective repair of meiotic heteroduplex DNA in the *msh6-FFAA,Δ51-251* mutant, there was no detectable impairment of MutSα antirecombination activity. Given the small, 10-fold range over which antirecombination can be measured in the IR assay, however, we cannot rule out a minor role of PCNA in regulating recombination fidelity. The relatively weak antirecombination defects conferred by the *pol30-201* and *pol30-52* alleles would be consistent with such a minor role, but they also could reflect the collateral effects of these alleles on DNA metabolism. We suggest that the *msh6-FFAA,Δ51-251* allele is a separation-of-function allele that distinguishes PCNA-dependent repair activities of the yeast MMR machinery (*i.e.*, the repair of mismatches in replication and recombination intermediates) from the antirecombination activity of the yeast MMR machinery, which our data suggest is largely, if not completely, PCNA independent. While this interpretation assumes that the functional redundancy of the Msh6 PIP domain with the region between residues 51 and 251 reflects PCNA-specific interactions, it is possible that redundancy might instead reflect alternative modes of localizing MMR proteins to regions of active DNA synthesis (see Shell *et al.* 2007 for further discussion and also Clark *et al.* 2007).

**Implications of the PCNA requirement for the MMR-dependent processing of recombination intermediates:** The MMR machinery detects and removes replication-generated mismatches in a reaction that is facilitated by PCNA and hence likely takes place in close proximity to the replication fork. In current models of recombination, invasion of a duplex DNA is followed by DNA synthesis primed from the invading 3′ end, a reaction that likewise requires a PCNA-tethered DNA polymerase. If the MMR-directed removal of mismatches in heteroduplex recombination intermediates occurs after extension of the invading 3′ end begins, one might expect an involvement of PCNA that is similar to that seen during replication. The meiotic data presented here is consistent with such a model, with disruption of the Msh6–PCNA interaction resulting in elevated PMS among non-Mendelian tetrads. In contrast, the interaction of the MMR machinery with PCNA appears to be of relatively little importance during antirecombination. This suggests that there may be a very early recombination-associated fidelity check that occurs during the initial strand-invasion process, *before* DNA synthesis is initiated from the invading 3′ end. Antirecombination data obtained using a transformation-based gap-repair assay indeed suggest that the blockage of recombination is separable from the repair of mismatches in recombination intermediates. In the gap-repair assay, recombinants produced in the presence of MMR show no evidence of persistent heteroduplexes (C. Welz-Voegele and S. Jinks-Robertson, unpublished data), indicating that intermediates can escape antirecombination activity of the MMR machinery and still be subject to mismatch correction. An early editing step during recombination might involve an Sgs1-mediated unwinding of the invading 3′ end, as Sgs1 is involved in antirecombination but not in mismatch correction (Myung *et al.* 2001; Spell and Jinks-Robertson 2004b; Sugawara *et al.* 2004). Once DNA synthesis initiates, however, we speculate that the MMR machinery switches to a repair mode, thus making the repair of mismatches in recombination intermediates mechanistically very similar to the repair of replication errors. We note that a two-stage model also has been proposed to explain antirecombination in *E. coli* (Stambuk and Radman 1998). Whether the late repair process we propose can also become a mechanism of antirecombination may depend on the density of mismatches in recombination intermediates.

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